Phase I Trial and Pharmacokinetics of Aziridinylbenzoquinone (NSC 182986) in Humans

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ABSTRACT

2,5-Diaziridinyl-3,6-(carboethoxyamino)-1,4-benzoquinone (AZQ) is a rationally designed antitumor agent which possesses sufficient lipid solubility to allow central nervous system penetration as well as adequate aqueous solubility for drug formulation and administration. We have conducted a Phase I trial of AZQ in 40 previously treated patients with advanced cancer. The drug was given as a 15-min i.v. infusion on Days 1 and 8 of a 28-day cycle. Seven dose levels ranging from 1 to 25 mg/sq m were studied with 3 to 11 patients treated at each level. Sixty-nine evaluable cycles of AZQ were administered. The major toxicity was myelosuppression, with the nadir in white blood cells and/or platelet count occurring at Days 15 to 20 of the cycle and first appearing at doses greater than 10 mg/sq m. The highest tolerated dose was 20 mg/sq m, and this dose is recommended for Phase II trials. Other toxicities were mild nausea, slight alopecia, and anemia. Plasma pharmacokinetics was studied in 11 patients by a high-performance liquid chromatography assay. Plasma decay curves could be fitted to a two-compartment open model of drug disappearance with a dose-independent terminal half-life of 33.3 ± 4.5 (S.D.) min. Cerebrospinal fluid AZQ levels were determined in three patients and revealed readily detectable levels of AZQ.

INTRODUCTION

AZQ2 is one of a class of new antitumor agents characterized by lipid solubility sufficient to allow central nervous system penetration and by aqueous solubility adequate for drug formulation and administration (3, 9).

Although its mechanism of action is presently unknown, the structure of AZQ (Chart 1) suggests that it may possess alkylating activity. Compounds of its class are also known to cross-link DNA, and AZQ has been shown to inhibit DNA synthesis in cultured cells with little effect on RNA or protein synthesis (1).

In animals, AZQ has a broad spectrum of antitumor activity. It inhibits the growth of B16 melanoma, colon 26, colon 28, CD8F mammary, and L1210 and P388 tumors (3, 4, 9). It is particularly interesting that i.p. administration of AZQ has produced significant increases in life span and some cures in the ependymoblastoma, intracranial L1210, and intracranial P388 model systems (3, 4, 9).

Preclinical toxicology studies carried out in mice, dogs, and monkeys demonstrated the primary sites of AZQ toxicity to be lymphoid tissue, bone marrow, and gastrointestinal tract (7). The incidence and severity of all toxicities were directly related to the administered dose. On the basis of the preclinical toxicity, a Phase I clinical trial in humans was begun. This paper reports the results of that trial along with initial pharmacokinetic studies of AZQ.

MATERIALS AND METHODS

Patient Selection. Patient characteristics are shown in Table 1. Forty patients, 28 men and 12 women, ranging in age from 20 to 68 years were entered on the study. All patients had pathological confirmation of cancer, and all but one patient had been treated previously with chemotherapy, radiotherapy, or combined modalities. Each patient had an initial complete history and physical examination, chest X-ray, electrocardiogram, urinalysis, complete blood count, and determination of serum chemistries. Complete blood count and platelet counts were then determined weekly during therapy while all other parameters were repeated on Day 1 of each cycle. Prior to beginning AZQ therapy, all patients had normal renal and liver function (serum creatinine less than 1.8 mg/100 ml and total bilirubin less than 1.5 mg/100 ml), WBC greater than 3000 cells/cu mm, and platelets greater than 100,000/cu mm. All patients gave written informed consent for participation in this study.

Drug Formulation and Administration. AZQ was provided in 10-ml vials containing 10 mg of drug. This was dissolved in 0.5 ml of N,N-dimethylacetamide and 9.5 ml of 0.01 M phosphate buffer, pH 6.5, to obtain a final concentration of 1 mg/ml. The prescribed dose was further diluted in 150 ml 0.9% NaCl solution and was administered i.v. over 15 min on Days 1 and 8 of a 28-day cycle. The initial dose of AZQ was 1 mg/sq m, one-third of the lowest toxic dose in large animals (2).

Dosage escalation was initially carried out according to a modified Fibonacci scheme; however, information obtained from other investigators at the Phase I Working Group Meeting (11) indicated that a more rapid dose escalation could be tolerated. At least 3 patients were treated at each dose level before dose escalation was carried out. There was no dose escalation in individual patients. Patients were treated for at least 2 cycles of therapy (4 doses) unless rapid disease progression or unacceptable toxicity occurred.

Pharmacological Studies. AZQ levels were measured in plasma, urine, and CSF using a HPLC assay described previously (8). Samples of venous blood were drawn into 10-ml heparinized tubes prior to initiation of the drug infusion and at predetermined times after completion of the infusion. The blood samples were placed on ice until centrifugation for 10 min at 2000 rpm in a Servall RC2 centrifuge (Dupont Instruments, Wilmington, Del.). The plasma was then decanted, frozen in dry ice, and stored at −20° until analysis.

A single 5-ml chloroform extraction was used to isolate and concentrate AZQ. Recovery of parent drug from normal plasma and urine, to which expected in vivo concentrations of AZQ (22 to 880 ng/ml) had been added, was greater than 88%. Following evaporation of the chloroform extract to dryness under N2, the residue was resuspended in 0.5 ml of 25% CH3CN-H2O. One hundred-μl aliquots were analyzed on a Waters Model 204W liquid chromatograph (Waters Associates,
was fit to the biexponential function representing a 2-compartment open model by using MLAB, an on-line computer modeling laboratory utilizing an iterative nonlinear least-squares regression program (10). Pharmacokinetic parameters were then calculated using standard equations incorporating infusion time outlined by Gibaldi and Perrier (5).

RESULTS

Toxicity. Forty patients were treated with AZQ during this study. Two patients died due to disease progression prior to the completion of a full cycle of therapy and are therefore not evaluable. One patient failed to return for follow-up studies following AZQ administration and is also considered unevaluable. The remaining 37 patients received 82 cycles of AZQ. Six cycles were not evaluable because of early death or disease progression. Seven cycles were excluded from analysis because they were administered at reduced doses necessitated by the occurrence of severe hematological toxicity during a prior cycle of therapy. Thus, 37 patients and 69 cycles of AZQ are evaluable for toxicity.

The major clinical toxicity observed in this trial was myelosuppression. As shown in Table 2, bone marrow toxicity first became apparent at doses as low as 10 mg/sq m. Dose escalation was associated with more profound nadir counts, such that at 17.5 mg/sq m significant bone marrow suppression (WBC less than 3,000 cells/cu mm or a platelet count less than 100,000 cells/cu mm) occurred in 67% of evaluable cycles of therapy. At 20 mg/sq m, however, depression of WBC and platelet count occurred less commonly (44% of evaluable cycles). As a group, patients treated at 20 mg/sq m did not differ significantly in age, performance status, or extent of prior therapy from other patients on study, and an explana-

Milford, Mass.) equipped with a Model 440 UV absorbance detector with a 340-nm filter, a U6K injector, and a Model 660 solvent program mer. A 5-fim Ultrasphere octadecylsilane column (4.6 x 250 mm; Altex Scientific Company, Berkeley, Calif.) was isocratically eluted with 25% CH3CN-H2O at 1 ml/min. The AZQ peak occurred regularly at 8.0 min and had a capacity factor (k' of 4.90 (Chart 2).

A standard curve for AZQ in plasma and urine was prepared by the addition of known amounts of AZQ to normal plasma and urine. The plasma standard curve was used for CSF because previous work had shown that recovery of AZQ from these biological fluids was similar (8). A new standard curve was generated for each batch of samples analyzed. Five µl of 4.9 x 10^-5 M 2,5-diamino-3,6-dichloro-1,4-benzoquinone in 25% CH3CN-H2O were added to both standards and unknown samples as an internal standard.

Peak areas and peak heights were determined simultaneously on a SP4100 computing and recording integrator (Spectra-Physics, Santa Clara, Calif.), and either could be used for quantitation. The standard curve was defined by least-squares regression analysis of the ratio of AZQ peak area or height to that of the 2,5-diamino-3,6-dichloro-1,4-benzoquinone internal standard versus known AZQ concentration. This ratio was calculated for unknown samples, and the AZQ concentration was determined from the appropriate standard curve. Correlation coefficients for standard curves were greater than 0.998. The limit of quantitation of the assay was 5 ng/ml, although smaller amounts of AZQ could be detected.

The measured AZQ concentration versus time curve for each patient
tion for their greater tolerance of the drug is not readily apparent. The 2 patients treated at 25 mg/sq m had malignant gliomas and had been treated previously with whole-brain radiation therapy only. Both patients experienced significant thrombocytopenia and mild leukopenia during at least one cycle of therapy.

The median blood count nadir occurred at Days 15 to 18 with complete recovery by Day 28 in most patients. In 6 patients (treated at 10, 15, 17.5, and 20 mg/sq m), blood count nadirs were prolonged, and subsequent therapy was delayed 1 to 2 weeks to allow recovery. These patients subsequently received reduced doses of AZQ.

Other toxicities associated with administration of AZQ are shown in Table 3. Mild nausea lasting 3 to 4 hr after drug administration occurred sporadically and was rarely associated with emesis. Diarrhea and mild alopecia occurred in an occasional patient. Of greater importance was the occurrence of anemia (unexplained fall in hemoglobin of greater than 2 g/100 ml during a cycle of therapy) in a significant proportion of patients treated at doses greater than 15 mg/sq m. None of these patients had evident blood loss or hemolysis as determined by Coomb’s tests, serum haptoglobin, and serum bilirubin, and their anemia is presumably due to suppression of the erythron by AZQ. There was no evident hepatic, renal, or central nervous system toxicity.

Responses. No patient had a complete or partial response to AZQ therapy. Two of 4 patients with malignant gliomas had objective neurologic improvement by physical examination. Minor tumor regression (less than 50% decrease in tumor volume) was also documented by computerized axial tomography scan in one of these 2 patients. The response of this patient to AZQ therapy lasted for 9 months. A third patient with adenocarcinoma of the lung metastatic to brain also had minor tumor regression demonstrated by brain computerized axial tomography scan but had no demonstrable clinical improvement. Finally, one patient with adenocarcinoma of the lung had a mixed response with a 50% decrease in the size of pleural-based masses but with progressive disease at other metastatic sites.

Pharmacological Studies. AZQ pharmacokinetics were determined in 11 patients at drug doses ranging from 1 to 20 mg/sq m. Chart 3 depicts a typical plasma decay curve in which the data points shown are for a patient treated at 10 mg/sq m. In all patients, plasma decay curves were remarkably similar with a very rapid redistribution phase followed by a slower but still fast elimination phase. For patients receiving 15 to 20 mg/sq m, AZQ plasma concentrations of 2 to 3 μg/ml were measured immediately following infusion. These fell to about 200 ng/ml by 1 hr after infusion and reached 50 to 75 ng/ml after 2 hr.

A summary of the pharmacokinetic parameters for the 11 patients studied is presented in Table 4. The mean elimination half-life (t1/2 β) was 33.3 ± 4.5 min and was dose independent. The plasma area under the curve was directly proportional to dose; a least-squares linear regression analysis of AZQ dose (mg) and area under the curve yielded a correlation coefficient of 0.94. AZQ total body clearance ranged from 294 to 815 ml/min and was dose independent, implying linear drug kinetics. The apparent volume of distribution at steady state averaged 15.8 ± 4.0 liters, suggesting a distribution only slightly less than the total extracellular water compartment.

The urinary excretion of unchanged AZQ was examined in 5 patients. Measurable amounts of intact drug were found in only 2 patients. The AZQ concentration in the initial 2-hr urine of a patient receiving AZQ, 10 mg/sq m, was 124 ng/ml; for a patient receiving AZQ, 20 mg/sq m, the level of unchanged drug appearing in the urine collected for 1 hr following the drug infusion was 241 ng/ml. For both patients, the amount of intact AZQ in the urine represented less than 0.2% of the administered dose of the drug. No AZQ was found in the urine of 3 other patients who had received either 17.5- or 20-mg/sq m doses of AZQ, even though the urine was collected in the first 2 hr after drug infusion.

A more crucial question was whether AZQ was able to enter the central nervous system. CSF was obtained via lumbar puncture from 3 patients. For one of these patients, there were 2 samples obtained on different days and treatment cycles. As shown in Table 5, AZQ was easily detectable in all CSF samples with CSF/plasma ratios of 1.4, 0.54, 0.21, and 0.55.

DISCUSSION

The major toxicity of AZQ given on Days 1 and 8 every 28
favorable experience in treatment of some patients with malignant gliomas and metastatic brain tumors is encouraging. Two of these patients, there was no clear-cut evidence for cumulative bone marrow toxicity following AZQ treatment, it seems highly unlikely that prior nitrosourea chemotherapy contributed in a disproportionate fashion. Unfortunately, only 5 evaluable patients received 3 or more cycles of AZQ chemotherapy. For these patients, there was no clear-cut evidence for cumulative bone marrow toxicity, although additional patients will need to be followed for multiple cycles of chemotherapy before it is certain that a cumulative effect does not occur. The drug was otherwise tolerated extremely well by all patients, with nausea, vomiting, diarrhea, and alopecia occurring infrequently and being of only minor consequence.

Although no complete or partial responses were noted, our favorable experience in treatment of some patients with malignant gliomas and metastastic brain tumors is encouraging. Two of 4 patients with deteriorating neurological status manifested dramatic improvement in neurological deficits after one cycle of AZQ. One of these patients had some tumor regression documented by computerized axial tomography scan and tolerated AZQ without difficulty for 9 cycles of therapy. We believe that Phase II trials of AZQ should be conducted particularly in patients with malignant gliomas and metastatic brain tumors and recommend a starting dose of 20 mg/sq m given on Days 1 and 8 every 28 days.

The HPLC assay for AZQ used in this study is specific for unmetabolized drug and has a limit of quantitation of 5 ng/ml for human plasma, urine, and CSF (8). The sensitivity of the assay was more than adequate to measure AZQ easily in all postinfusion samples obtained after doses of 5 to 20 mg/sq m. Indeed, if plasma samples had been available, the terminal elimination phase of the drug could have been followed for at least 2 additional half-lives after doses of 10 to 20 mg/sq m.

The plasma disappearance curve of AZQ could be fitted to a 2-compartment open model for all 11 patients, although it is possible that a third component of drug disappearance might have been identified if plasma sampling had been carried beyond 2 hr postinfusion.6,4 The observed mean half-life of the terminal elimination phase is nearly identical to that noted in rats (8) and dogs5 and is quite similar to that reported for chloroform-extractable radioactivity in rhesus monkeys given [14C]AZQ (6). This, coupled with the unusually narrow range of the \( t_{1/2B} \) values in this study and the linear kinetics displayed by the drug, suggests that the plasma disappearance of the parent drug proceeds by similar mechanisms in these species. A mean total body clearance of 517 ± 155 (S.D.) ml/min implies processes other than renal clearance as the primary mechanisms of AZQ elimination. Thus, spontaneous drug decomposition or hepatic metabolism to as yet unidentified compounds may well be important routes of drug elimination.

AZQ clearly enters the CSF and achieves concentrations which are substantial compared to plasma levels. This confirms that the drug acts as intended since its experimentally determined octanol-water partition coefficient predicts almost equal distribution into both phases. Since only one time point was available from each patient studied, it is difficult to speculate on the shape of the CSF concentration-versus-time curve. Certainly, CSF AZQ levels in humans are comparable to those observed in rhesus monkeys (6), in which peak CSF levels occur 60 to 100 min after completion of the drug infusion. A characterization of CSF pharmacokinetics must await further studies.

Table 4
Pharmacokinetic parameters of AZQ derived from a 2-compartment open model

<table>
<thead>
<tr>
<th>Patient</th>
<th>AZQ dose (mg/sq m)</th>
<th>( t_{1/2A} ) (min)*</th>
<th>( t_{1/2B} ) (min)</th>
<th>Area under the curve (µg-min/ml)</th>
<th>Apparent total body clearance (ml/min)</th>
<th>Apparent volume of distribution at steady state (liters)</th>
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<tr>
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<td>17.5</td>
<td>3.3</td>
<td>37.1</td>
<td>68.0</td>
<td>499</td>
<td>17.3</td>
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<tr>
<td>11</td>
<td>20</td>
<td>4.2</td>
<td>35.2</td>
<td>69.0</td>
<td>623</td>
<td>20.7</td>
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</table>

\[ 2.8 ± 1.3^{5} \]
\[ 33.3 ± 4.5 \]
\[ 517 ± 155 \]
\[ 15.8 ± 4.0 \]

* \( t_{1/2A} \), half-life of initial redistribution phase; \( t_{1/2B} \), elimination half-life for terminal phase.

Table 5
CSF levels of AZQ

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose (mg/sq m)</th>
<th>Time (min)</th>
<th>[AZQ](_{\text{plasma}}) (ng/ml)</th>
<th>[AZQ](_{\text{CSF}}) (ng/ml)</th>
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<td>11</td>
<td>20*</td>
<td>95</td>
<td>110*</td>
<td>61</td>
</tr>
</tbody>
</table>

* Day 1, cycle 1.

* Day 6, cycle 3.

* Calculated from the pharmacokinetic curve.

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Current studies of AZQ pharmacokinetics in plasma and CSF indicate that a third phase is highly unlikely. AZQ can be measured in plasma for 4 hr postinfusion after a dose of 20 mg/sq m with the plasma disappearance curve fitting a 2-compartment open model in all cases. In no case was the drug detectable in plasma samples that were obtained more than 6 hr postinfusion.


E. D. Siu Chong and J. M. Strong, unpublished data.
tion and analysis of serial CSF samples. This Phase I study confirms that AZQ, a drug which has been rationally designed to enter the central nervous system, does indeed achieve this goal at tolerable systemic doses. Its value in the treatment of primary and metastatic brain tumors should be further investigated.

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